OBSERVATIONS ON THE RELATIVE CONSTANCY OF AMMONIA PRODUCTION BY CERTAIN BACTERIA.*

STUDIES IN BACTERIAL METABOLISM. X.

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Experiments were reported in a series of previous communications[1] which were designed to measure quantitatively the nature and extent to which utilizable carbohydrate protects protein or protein derivatives from bacterial breakdown. The principle upon which this sparing action of carbohydrate for protein depends is of more than academic interest: it plays a prominent part in many practical as well as theoretical fields in which bacterial activity is involved. This principle, which is fundamentally concerned in the metabolism of utilizable carbohydrate and protein by bacteria, may be stated thus: "Fermentation takes precedence over putrefaction,"[2] that is to say, those bacteria which can utilize both carbohydrate and protein utilize the former in preference to the latter when both are simultaneously available. It should be remembered in this connection that some protein must be available, even when carbohydrate is being thus acted upon, since bacteria in common with all known cellular organisms contain nitrogen in their substance. Carbohydrate contains no nitrogen, consequently carbohydrate alone is obviously unable to supply the nitrogen. Bacteria cannot grow in pure carbohydrate solutions. This nitrogen requirement is minimal when bacteria are metabolizing carbohydrate. That it is indispensable, however, focuses attention sharply upon two entirely distinct activities which the bacteria, and indeed all living cells, exhibit, the structural (anabolic) and the vegetative (katabolic) functions. The structural function precedes the vegetative function chronologically, for the cell must be formed before it can carry on its appropriate functions. The

* Received for publication June 18, 1913.

[1] Kendall and Farmer, *Jour. Biol. Chem.*, 1912, 12, pp. 13, 215, 219, 465; *ibid.*, 13, p. 63.

[2] Kendall, *Jour. Med. Research*, 1911, 25, p. 117.

actual amount of material involved in the structural process is very little; it can be estimated with a fair degree of accuracy. The following simple calculation indicates the manner in which this estimation is reached. The colon bacillus is approximately one micron in diameter and two microns long. Its volume is, consequently, one micron $\times$ one micron $\times$ two microns. Assuming for simplicity of calculation that it is a rectangular prism instead of a cylinder, the volume of a colon bacillus is $0.001 \times 0.001 \times 0.002$ mm. The specific gravity of the colon bacillus has been estimated at 1.038, hence the weight of a single colon bacillus would be theoretically: $0.001 \times 0.001 \times 0.002 \times 0.038$, or 0.000,000,0021 mg. That is to say, two thousand million colon bacilli would weigh about one milligram. Eighty-five per cent of this weight is water. Hence the actual weight of organic material is inconceivably small. It should be remembered in this connection that the structural requirement, aside from losses incidental to enzyme formation, etc., which must be replaced, is ended when the colon bacillus is morphologically complete. On the contrary the requirements for vegetative activity, the "fuel" requirement in other words, is a continuous one which ceases only when the organism dies or is placed in such an environment that all vital functions are suspended, as for example, exposure to cold. The waste from structural needs is inconspicuous and is usually lost sight of in discussing bacterial metabolism for it is overshadowed by the relatively prominent phenomena incidental to the fuel waste. The results of the vegetative activities are those associated with the utilization of "fuel" rather than structural materials. Purely qualitative observations upon bacterial activity, therefore, cannot be relied upon to furnish a complete survey of all the processes involved and yet the recognition of these processes is practically important wherever bacterial activity is concerned, be it in medicine, agriculture, manufacturing, or in the preparation of bacterial products. A brief résumé of certain striking instances where bacterial metabolism plays a prominent part has been published previously.[1] It is apparent that a more definite knowledge of the actual chemical reactions involved in metabolism will be advantageous both to

[1] Kendall, *Jour. Med. Research*, 1911, 25, p. 117; *Boston Med. and Surg. Jour.*, 1913, 168, p. 825.

explain the observed changes which bacteria bring about and to serve as a theoretical basis upon which to erect a rational and specific method for correcting them.

The study of bacterial metabolism, furthermore, may confidently be expected to throw a certain amount of light upon cellular metabolism in general, for it must be conceded that the metabolism of the human body reduced to its lowest terms is unicellular metabolism, modified along special lines to meet certain necessary physiological divisions of labor.

The metabolism of a certain number of bacteria has been studied chemically, using methods which have been described in detail in earlier publications.[1] The results of these experimental metabolic studies which were concerned chiefly with the utilization of carbohydrate and protein were suggestive, and fairly definite. Briefly they appear to substantiate the theory that "fermentation takes precedence over putrefaction," at least for many bacteria of interest in medicine. The question naturally arises: can these results be fairly duplicated at any time, and with any strain of the organism in question? Table 1 contains the analytical data for *B. typhosus* and *B. coli* which have been studied in the Northwestern University Medical School, using the same methods and strains of organisms as those referred to above.

This holds true only for broths made from the same type of ingredients using a uniform technic of preparation throughout. Broth made from meat extract and peptone would give consistently different results from similar broth made from meat juice and peptone; these differences furthermore become more and more marked as the protein constituents of the media become more and more simple in their structure. The substitution of amino acids for peptone would bring about an entirely new readjustment of proteolytic values. On the other hand, standard nutrient broth made in accordance with a definite formula will give fairly concordant results, provided the cultures are of maximum vegetative activity. This is shown in the table.

It is reasonable to expect that physiological limits may be determined for various groups of bacteria in terms of quantitative

[1] *Loc. cit.*

chemical data and that unusual variants which differ from their respective types shall be scrutinized from entirely new points of view. Such chemical procedures will not supplant biological

TABLE I.

	Plain Broth		Dextrose Broth	
	Free NH_3 as mgs. N_2 per 100 c.c. Broth	$\frac{\text{Ammonia Nitrogen}}{\text{Total Nitrogen}}$	Free NH_3 as mgs. N_2 per 100 c.c. Broth	$\frac{\text{Ammonia Nitrogen}}{\text{Total Nitrogen}}$
		per cent		per cent
B. coli I. *a*..........	19.60	+5.01	+4.20	+0.53
" " *b*..........	16.10	+5.35	+1.40	+0.29
" " II. *a*..........	21.10	+6.74	+0.70	+0.29
b..........	21.70	+7.58	0.00	0.00
" " III. *a*..........	17.50	+7.80	+1.40	+0.64
b..........	22.40	+7.80	0.00	0.00
B. typhosus I. *a*.....	7.00	+2.89	+1.00	+0.58
b.....	8.40	+3.58	+0.70	+0.25
" " II. *a*.....	8.75	+2.60	−3.50	−1.70
b.....	4.90	+1.63	−0.70	−0.26
" " III. *a*.....	3.50	+1.68	+2.55	+1.27
b.....	6.00	+2.20	+1.40	+0.50
" " IV. *a*.....	4.55	+2.13	+2.80	+1.33
b.....	5.60	+2.05	+1.40	+0.50
" " V. *a*.....	10.00	6.65	0.00	0.00
b.....	10.50	3.79	+0.35	+0.13

The horizontal columns "*a*" and "*b*" indicate respectively the amounts of ammonia produced by various strains of *B. coli* and *B. typhosus* respectively in standard nutrient broth, with and without one per cent of dextrose in Boston and Chicago. Column "*a*" represents the values obtained in Boston; column "*b*" the corresponding values obtained in Chicago eighteen months later, using the same strains.

reactions such as agglutination tests for purposes of identification, but it may be confidently predicted that quantitative measurements of bacterial activity will be of material value in the ultimate reduction of this activity to a definite chemical basis.